EXPRESSION OF HDAC1 IN LEUKEMIA CELLS

Thesis Submitted To

National Institute Of Technology, Rourkela

For Partial Fulfillment

Of The Master Of Science degree In Life Science

Submitted by

HIMANI SETHI

ROLL NO – 410LS2074

Under the guidance of

Dr. SAMIR KUMAR PATRA

ASSOCIATE PROFESSOR AND HEAD

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY
ROURKELA-769008, ODISHA

2011-2012

DEPARTMENT OF LIFE SCIENCE
NATIONAL INSTITUTE OF TECHNOLOGY,
ROURKELA-769008

.....

Dr. Samir KumarPatra Ref. No.

Associate Professor and Head. Date: .....

CERTIFICATE

This is to certify that the thesis entitled "EXPRESSION OF HDAC IN LEUKEMIA CELLS" which is being submitted by Miss Himani Sethi , Roll No.410ls2074 , for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by her under my supervision. The results embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

Dr. SAMIR K. PATRA

ASSOCIATE PROFESSOR AND HEAD,
Department of Life Science
National Institute of Technology

Rourkela – 769008, Odisha, India.

.....

Phone no: 0661-2462683. Email:skpatra_99@yahoo.com

DECLARATION

I, Himani Sethi, hereby declare that this project report entitled **"**Expression of HDAC1 in Leukemia cells**"** is the original work carried out by me under the supervision of Dr. Samir K. Patra, Associate Professor and Head, Department of Life Science, National Institute of Technology, Rourkela. To the best of my knowledge and belief the present work or any other part thereof has not been presented to any other University or Institution for the award of any other degree.

Date: 08.05.2012 **HIMANI SETHI**

Place: NIT ROURKELA

ACKNOWLEDGEMENT

I wish to express my deepest sense of gratitude to my supervisor Dr. Samir K. Patra, Associate Professor, Department of Life Science, National Institute of Technology, Rourkela for his valuable guidance, assistance and time to time inspiration throughout my project.

I am very much grateful to Prof. S. K. Sarangi, Director, National Institute of Technology, Rourkela for providing excellent facilities in the Institute for carrying out research.

I would like to take the opportunity to acknowledge quite explicitly with gratitude my debt to Dr. Suman Jha, Dr. Vivek Mallick, Dr. Bismita Nayak, Dr. Surajit Das and Staff, Department of Life Science, National Institute of Technology, Rourkela for his encouragement and valuable suggestions during my project work.

My heartfelt thanks to Ms. Moonmoon Deb, all the other PhD scholars and second yr. friends for their inspiration and support throughout my project work

Finally I was highly great full to my parent for their continual moral support.

And to all mighty, who made all things possible……..

ABSTRACT

Histone deacetylases (HDACs) is an enzyme which removes the acetyl group and thus completes the reversible modification of histone by acetylation. DNA in a chromatin is very tightly associated with proteins called histones, which packages in nucleosomes also found in chromatin are many nono histones proteins, some of which help maintain chromosomes structures and others that regulate the expression of specific genes. Histones are found in all eukaryotic cells having molecular weights between 11,000 and 21,000. They are rich in amino acids like arginine and lysine. Histones are subjected to enzymatic modification like methylation, acetylation, ADP-ribosylation, phosphorylation, ubiquitination. Histone acetylation and deacetylation are two regulatory and opposing mechanism in leukemia cells. HDACs are major groups for causing repression of tumour suppressor genes and hence they cause cancer. So HDACs can be a proved a promising target for curing cancer.

Keywords

- Histone deactylases, cancer, ubiquitination, ADP-ribosylation

CONTENTS

LIST OF FIGURES

LIST OF TABLE

ABBREVIATIONS

ABBREVIATIONS	FULL FORM
β	Beta
µ	micro
°	degree
M	Molar
g	gram
min	minutes
sec	seconds
HDAC	Histone deacetylases
l	litre
nm	Nano meter
1U	One unit

1. Introduction

Histone acetylation – attachment of acetyl groups (-$COCH_3$) to certain amino acids of histone proteins; Histone deacetylation– the removal of acetyl groups (Fig.1).

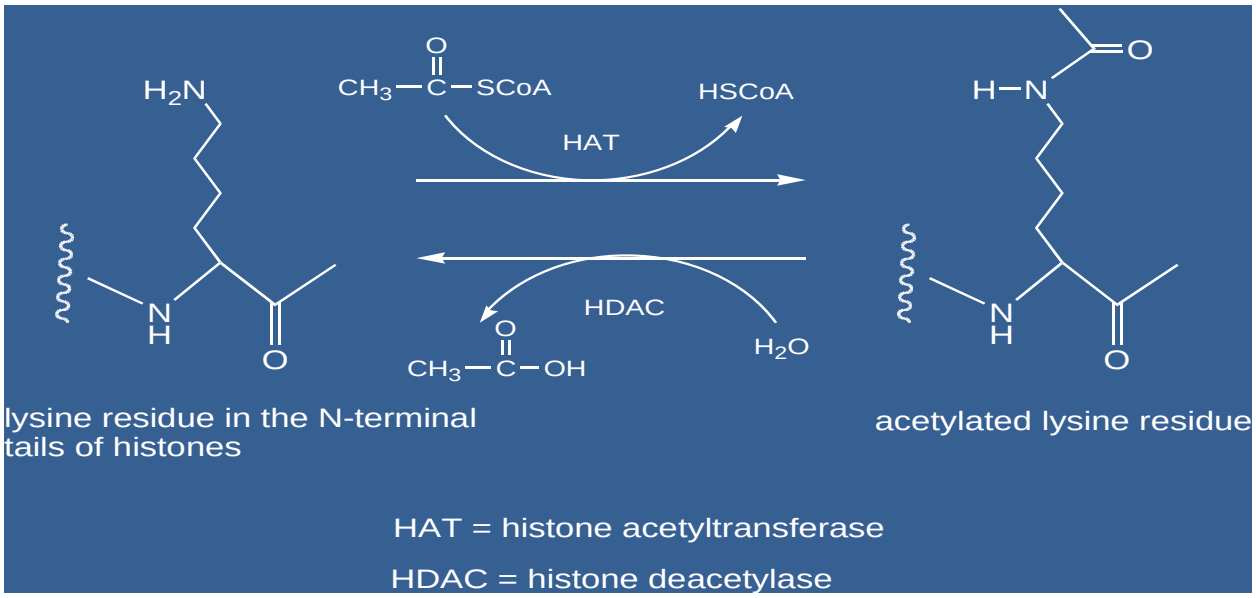

Fig.1 Mechanism of addition and removal of acetyl group from histones (Yang et al, 2006)

HDACs are multiproteins complexes, like transcriptional corepressors mSin3, N-CoR, and SMRT (Glass and Rosenfeld, 2000). This methyl-binding protein complexes recruits HDAC-containing complexes to methylated gene promoters as a mechanism for gene-transcription repression (Jones et al., 1998) and(Nan et al., 1998).Histone deacetylases (HDAC) are a new class of targeted for cancer , hence inhibition to HDACs mechanism may be proved as new class of inhibitors to cancer they will be used as anticancer agents which are inducers of growth arrest, differentiation, and causes apoptotic cell death of cancer cells *vitro* and *in vivo*.. HDAC inhibitors are those which selectively altered the expression of genes. They hindered in pathway of HDACs mechanism. There are 4 class of HDAC inhibitors-short chain fatty acids (4-phenylbutyrate and valporicAcid), Hydroxmic acid, (suberoylanilidehydroxamic acid (SAHA), pyroxamide, trichostatin A (TSA), cyclic tetra peptides (trapoxin, apicidin). These can induce cancer cell death, It has been showed that HDAC inhibitors could be used to induce differentiation of leukemia cells or solid tumor cells, for example sodium butyrate and trichostatin A (TSA) could increase the remission rate of many kinds of tumors obviously by

cooperation with other anticancer drugs (4). There by were viewed the progress of histone deacetylases inhibitors used in treatment of tumor cells, with analysis of its mechanism. Epigenetic regulation of gene transcription is now being been the subject of a fast growing research area especially in the field of cancer. Histone deacetylases (HDACs) and histone acetyl transferees (HAT) are two families of enzymes that take part in the control of the acetylation level of the histone tails rich in amino acids(Marks P et al., 2001;Allfrey VG et al., 1964;de Ruijter AJ et al., 2003;Taunton J et al., 1996).HATs transfer acetyl groups to the amino group of an lysine residues located in the N-termini of the core histones. It leads to the expansion of chromatin and increased accessibility of regulatory proteins to DNA resulting in activation of gene transcription. Reversely, HDACs counteract the activity of HATs and remove the acetyl groups from lysine residues in histones. This results in chromatin condensation and transcriptional repression, this are essential fundamental cellular processes. The main function of HDACs are changing chromatin structure and gene transcription, repression of genes required for cell proliferation, regulation of gene expression by deacetylation transcription factors, gene silencing, differentiation, and participation in cell cycle regulation. In addition, HDACs can also acetylate and deacetylated transcription factors and other regulatory protein (Glozak MA et al., 2005; Fig.2). It has been suggest that deregulation of acetylation and deacetylation plays a causative role in the abnormal regulation of gene expression in many forms of cancer (Cress WD et al., 2000; Mahlknecht U and Hoelzer D., 2000; Timmermann S et al., 2001). Histone hypo-acetylation is the overexpression of HDAC and is directly related to the initiation and progression of various tumors. In contrast it was seen that histone modification like acetylation, deacetylation, methylation and ubiquanation have impact on leukemia cells. E-Cadherin, a transmembrane glycoprotein, is also a calcium-dependent cell–cell adhesion molecule, known to play a key role in the maintenance of tissue integrity by forming complex with catenin. E-Cadherin is eventually tagged to actin cytoskeleton through catenins. Because loss of E-cadherin expression results in disruption of cellular clusters, it has been postulated that E-cadherin functions as tumor suppressor gene. mRNA and protein expression of E-cadherin is frequently lost on DNA methylation in multiple cancers at the early stage of tumor progression.(Patra et al, 2001).HDACs removes the acetyl groups from amino acid rich histone tails and alters the gene transcription and leads to the repression of tumour suppresser genes that causes the proliferation of cancerous cells.

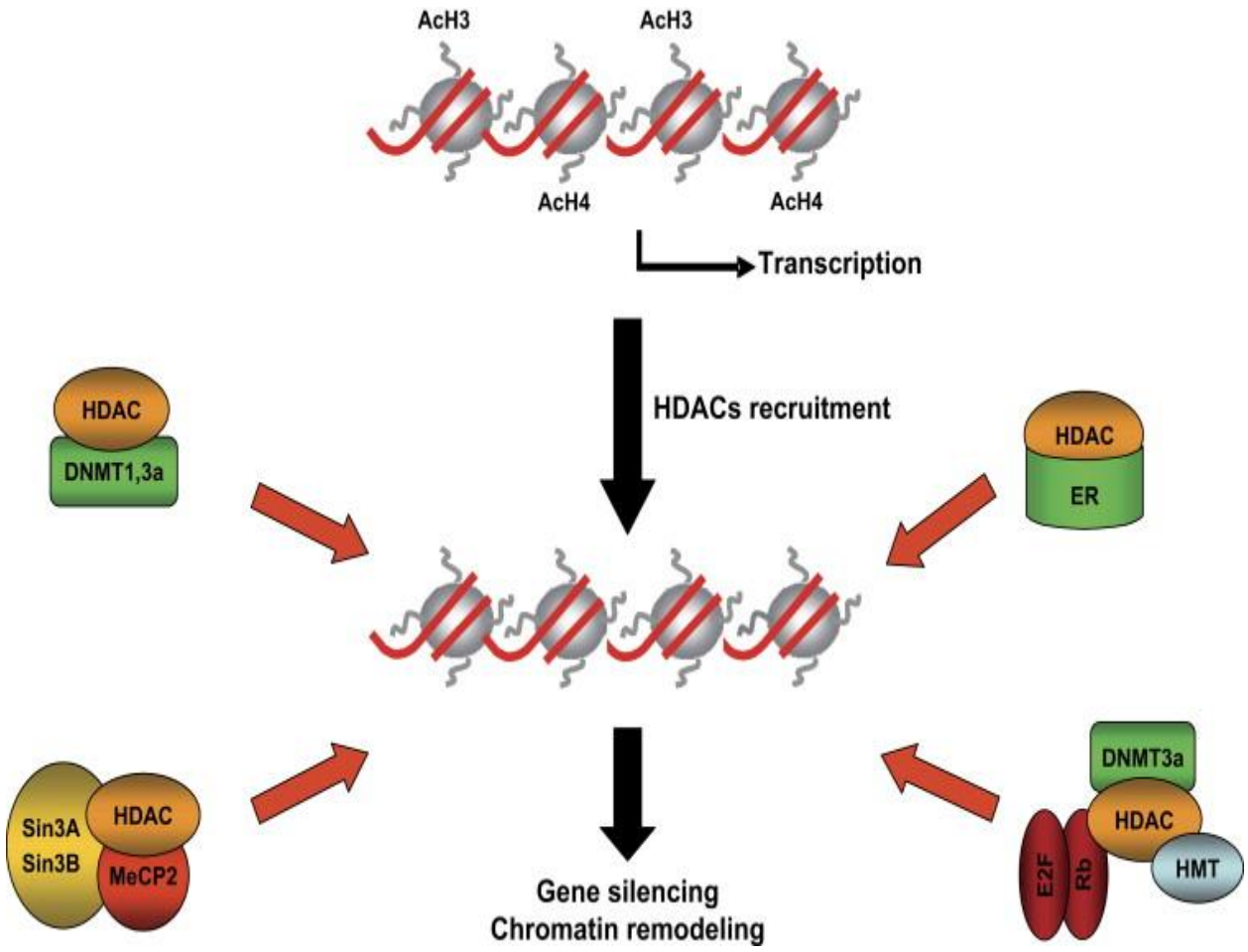

Fig.2 Different ways by which HDACs are recruited to gene promoters. An array of nucleosomes is shown. Histone octamers are represented by circles and the DNA is shown in red. Histone deacetylation induced by recruitment of HDAC to gene promoters by different factors including, DNA methyl transferases (DNMT), the methyl binding protein MeCP2, Estrogen receptor (ER) and transcription factors (E2F, Rb).(Javierre et al.,2008)

2. REVIEW OF LITERATURE

The transcription process is associated with structural changes in chromatin and the process is called chromatin remodeling. This is changes are brought by enzymes that covalently modifies the histones of nucleosomes. Acetylation and deacetylation are prominent in this process.

Histone deacetylases (HDAC) are involved in inducing cancer through their regulation of cell proliferation, differentiation. The inhibitors of HDAC exhibit profound effects in cancer treatment when combined with their anticancer drugs. The molecular mechanism underlying this are not fully understood. The acetylation and deacetylation of lysine in the amino terminal tails of core histones play important roles in the remodelling of chromatin and regulation of transcription. Histone deacetylases (HDAC) catalyse the removal of acetyl groups from histones that cause chromatin condensation and resulting in transcriptional repression. In addition to histones, HDACs target many other protein substrates and affect their functions in cell proliferation and cell death (Minucci S and Pelicci PG, 2006) and (Bolden JE et al., 2006). Mammalian HDACs are categorised into several classes based on their sequence similarity to yeast transcription gene. There are mainly four classes of HDACs- class –I HDACs, class II, class III, class –IV. Class IHDACs (HDAC1, HDAC2, HDAC3, and HDAC8) mainly are primarily nuclear in localization and expressed and act on multiprotein complexes (Yang XJ and Seto E., 2008). Aberrant expression of several class IHDACs in tumor and cancer samples has been reported (Halkidou K, et al., 2004)(Wilson AJ et al., 2006)(Zhang Z et al., 2005)(Huang BH et al., 2005)(Zhu P,et al., 2004)(Song et al., 2005)(Nakagawa M et al., 2007). The relationship between altered expression of HDACs and cancer progression remains largely correlative. However, RNA interference (RNAi)–mediated knockdown of individual HDACs, these are over expressed in certain tumor cell lines, and suppresses tumor cell growth and survival (Wilson AJ et al., 2006) (Huang BH et al., 2005),(Zhu P,et al., 2004), (Glaser KB et al., 2003), suggesting that over expression of HDACs could contribute to tumor induction and progression. In vitro studies have revealed that pharmacologic class I and IIHDAC inhibitors (HDACi) can arrest cell growth and induce terminal differentiation and cell death in transformed cells.The effect of these is primarily achieved by induction of anti-proliferative, prodifferentiative, or proapoptotic genes (Minucci S and Pelicci PG, 2006), (Bolden JE et al., 2006), (Xu WS et al., 2007). HDACi can selectively kill cancer cells and have limited toxicity for normal cells which is the basic requirement that it should not cause any deleterious effect on

normal cells. For this reason several HDACs inhibitor was undergoing clinical trials for their anticancer activity. One such inhibitor, suberoylanilidehydroxamic acid (SAHA), it has been approved for treatment of cutaneous T-cell lymphoma (Xu WS et al., 2007) and (Marks PA et al., 2007).Whereas their activity in preclinical models has been promising, HDAC inhibitors have shown relatively good antitumor activity against solid tumors in human clinical trials (Nolan L et,. 2008). More recently, their use in combination with other agents has suggested greater efficacy(Bolden JE et al., 2006);that is, HDACi have shown positive or additive effects with a wide variety of anticancer reagents, that includes conventional chemotherapeutic drugs (Bolden JE et al., 2006) and(Xu WS et al., 2007), leading to the suggestion that HDACi lower the apoptotic threshold of tumor cells. However, the mechanistic basis of the synergistic effect with anticancer agents is not fully understood.HDAC1 is found in multiprotein complexes, such as Sin3 (Hassig CA et al., 1997), nucleosome remodelling and deacetylases (Tong JK, et al., 1998), and corepressors of RE1-silencing transcription factor (Humphrey GW, et al., 2001), these two functions as the catalytic subunit. The commonness feature of these complexes is their interaction with sequence-specificDNA-binding transcription factors, that repressing transcription and cooperating with other chromatin modifiers to shape epigenetic programs (Grozinger C and Schreiber SL, 2002). HDAC1-mediated control of epigenetic regulation plays vital roles in normal development and tumor progression. For example, in normal development, disruption ofHDAC1 causes early embryonic lethality due to increased expression of p21(cyclin-dependent kinase inhibitor 1) (Lagger G, et al., 2002). In cancer cells the role of , HDAC1 is to repress the expression of tumor suppressive genes such as p21WAF1/CIP1 andBax (Lagger G, et al., 2003) and (Juan LJ et al., 2000), leading to aberrant cell proliferation and cell viability. Although it has been well shown and recorded that HDAC1 regulates the cell cycle as well as the differentiation and death of cells through its interactions with various proteins, less is known about the effects of HDAC1 on cellular sensitivity to stresses such as anticancer drugs, chromatin was inactivated by histone deacetylases this inhibit binding of transcription factors including RNA polymerase (patra et al,2001) HDACs are not only the cause of cancerous stage but over expression of DNMTs and hypermethylation of –CPG- islands at regulatory regions they participate for regulation of transcription in association with biological events including cancer development.(patra et al, 2003)

2.1 CLASSIFICATION OF HDAC FAMILY MEMBERS

HDACs are grouped into various classes like, class I, class II, class III and classIV which is based on their sequence homology to their yeast orthologuesRpd3, HdaI and Sir2, respectively (A.J. de Ruijter et al., 2003) and (I.V. Gregoretti et al., 2004)(Fig.3). Class I, II,and IV are referred to as ‘‘classical” HDACs and comprise11 family members , whereas class III members are named sirtuins (I.V. Gregoretti et al., 2004). Thier difference is based on their catalytic mechanisms. Classical HDACs are metal like Zn2+-dependent enzymes having a catalytic pocket in which Zn2+ ion is present at its base that is inhibited by Zn2+ chelating compounds such as hydroxamic acids. These compounds are not active against sirtuins as these classIII enzymes have a different mechanism of action that requires NAD+ (a cofactor) as an essential cofactor (A.J. de Ruijter et al., 2003). The term ‘‘HDAC inhibitors “is commonly used for compounds that target the‘‘classical” class I, II, and IV HDACs and that are currently evaluated in clinical trials.

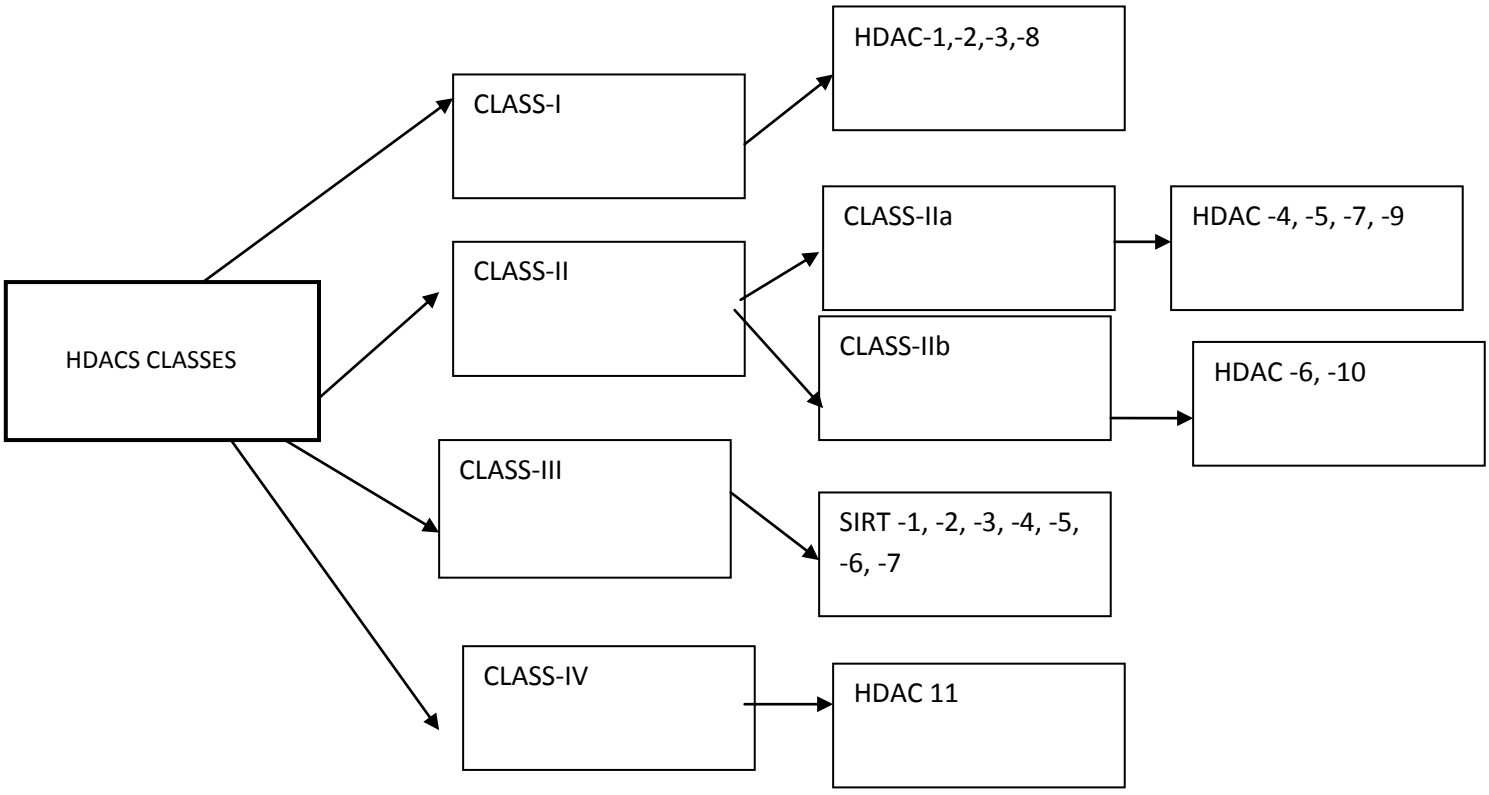

Fig.3. Classification of HDACs

The initially discovered and best studied enzymatic activity of HDACs is the deacetylation of histone proteins. Through this mechanism, HDACs control the interaction of positively charged

histones with the negatively charged DNA that is present in form of phosphate group , the HDACs inhibits the binding of amino acid with the phosphate group present in the DNA and thus regulate chromatin conformation and transcriptional activity. In general, high HDAC activity is observed with condensed, transcriptionally inactive chromatin. In surplus to this epigenetic function of HDACs, it is recognized that certain HDACs also exhibit important cytoplasmic function by controlling the acetylation status and function of numerous cytoplasmic proteins and transcription factors. Therefore, the more precise term to be used is for these enzymes would be ‘‘lysine deacetylases” as to show that their substrates are not restricted to histone proteins (X.J. Yang and E. Seto ,2008).

2.1.1 CLASS I HDACs

HDAC family members 1, 2 and 3 are constituent subunits of multiprotein nuclear complexes that are important for transcriptional repression and epigenetic functions. For example, HDAC1 and 2 are components of the HDAC-I complex which are responsible for the expression of neuronal genes in non-neuronal tissues (Y. Huang et al., 1999). Complexes containingHDAC1 and 2 are the NURD and SIN3 repressor complexes (J. Ahringer, 2000). HDAC3 is found within the N-COR and SMRT repressor complex (Y.D. Wen et al., 2000). It is to be noted that class I member HDAC8 has not been found to be a component of any repressor complex so far, itsuggest that a particular function for this class I HDAC.

2.1.2 CLASS II HDACs

Class II HDAC family members are further sub divided into IIA and IIB. Class IIA members HDAC4, 5, 7,9 are generally known by a large, functionally important N-terminal domain that regulates nuclear-cytoplasmic shuttling and specific DNA-binding. The nuclear import and export signals are regulated by cellular trafficking of these HDACs. They also act as binding site for 14-3-3 proteins. HDAC4, 5, 7, 9 has three conserved 14-3-3 binding sites. Cytoplasmic retention was induce by binding of the 14-3-3 proteins which in turn regulates the activity of transcription factors like the myocyte enhancing factor-2 (MEF2) (X.J. Yang and E. Seto ,2008)(T.A. McKinsey et al 2000)(E. Verdin et al 2000) Major signalling pathways, like Ca2+/calmodulin-dependent kinases (CaMKs)(T.A. McKinsey et al 2000), protein kinase-D

(R.B. Vega et al 2004), microtubule affinity-regulating kinases (S. Chang et al., 2005), salt-inducible kinases (R. Berdeaux et al., 2007)and checkpoint kinase-1 (CHK1) (M.A. Kim et al., 2007) regulate phosphorylation of these 14-3-3 binding sites kinases activity regulates phosphorylation of these binding sites. Class IIB HDAC6 has two deacetylase domains and a C-terminal zinc finger. HDAC6 hasa major cytoplasmic deacetylase functioning as ana-tubulin deacetylase(C. Hubbert etal., 2002) and HSP90 deacetylase(J.J. Kovacs et al., 2005) thereby it regulates the cell motility, adhesion and chaperone function. , HDAC6 exerts cellular functions that are independent from its deacetylase activity. Binding to ubiquitin through its zinc finger domain regulates aggresome function, autophagy, heat shock factor-1 (HSF-1) and platelet derived growth factor(PDGF) function (Y. Kawaguchi et al., 2003) and (U.B. Pandey et al., 2007). HDAC10 is structurally related toHDAC6, but it contains one additional catalytically inactive domain that make it differs from HDAC 6 structurally both are same but functions different in catalytic way .Its function is largely unknown til now.

2.1.3 CLASS III HDAC

HDAC-3 is a gene which encodes for enzyme histone deacetylases. It altrs chromosome structure and affects transcription factor. Protein encoded by this gene belongs to the histone acetylases/deacetylases family. It represses transcription. This protein down regulates p53 function and hence modulates cell growth and apoptosis. This gene was regarded as potential tumor suppressor gene.

2.1.4 CLASS IV HDAC

Class IV consists of only one that is HDAC11 only. It is structurally related to both, class I and II HDACs. Very few information is available about its expression and function. Major number of non-histone proteins like those includes, Stat3, HMGs, HSP90, NF-jB,p53, E2Fs, GATA1,Bcl-6 tubulin, importin, nuclear hormone receptors, and b-catenin are recognized as substrates of HDACs (for review see (M. Dokmanovic et al., 2007).For example, HDAC1 has been shown to regulate the activity of the transcription factor p53. p53 stability is reduced by deacetylases functions it also represses its its transactivation activity and interaction with DNA, and. This in turn modulates p53-mediatedcell growth arrest and apoptosis (W. Gu,and R.G. Roeder. 1997)

and (J. Luo et al., 2000). Thus, it can be said that HDACs regulate the activity of cellular key players that involves in regulation of transcription, cell cycle, apoptosis, signal transduction and others. This clearly shows that HDACs regulate important cellular functions that are independent from their epigenetic role in controlling chromatin structure and hence helps in chromatin remodelling.

2.2 HDAC FAMILY MEMBERS IN CANCER

HDACs have much function in regulation but, surprisingly little is known about the expression of their targets in cancer tissues. A recent study demonstrates resistance to HDAC inhibitors in cells lackingHDAC2 expression (S. Ropero et al., 2006). In this section, we review our current knowledge on the expression and function of the classical HDAC family members 1–11 in cancer cells. HDAC polymorphisms and cancer risk Germline variants of several HDACs have been studied leukemia patients. Neither study found evidence for association of HDAC3, 4 and 5 variants with lung cancer risk (J.M. Park et al., 2005), or HDAC2 and 5 with breast cancer risk (A. Cebrian et al.,2006). An insertion of a CAG triplet in the 50-UTR ofHDAC2 was recently identified in 18% of 181 cancer samples investigated versus 10% of 192 normal DNA controls($P < 0.01$) (H. Ozdag et al., 2006). HDAC10 promoter polymorphism in 24 patients with hepatocellular carcinoma (HCC) resulted in increased promoter activity in vitro and was associated with development of HCC among chronic HBV patients (B.L. Park et al., 2007)

2.3 SOMATIC HDAC MUTATIONS IN CANCER

Somatic mutations of the HDAC2 gene in human epithelial cancers with microsatellite instability have been identified in cell lines (S. Ropero et al., 2006) and (H. Ozdag et al., 2006). A HDAC 2 mutation was detected in 48/228 (21%) of investigated cancers with microsatellite instability in which there is loss of HDAC2protein expression (Fig.4). surprisingly, the mutation was viewed in

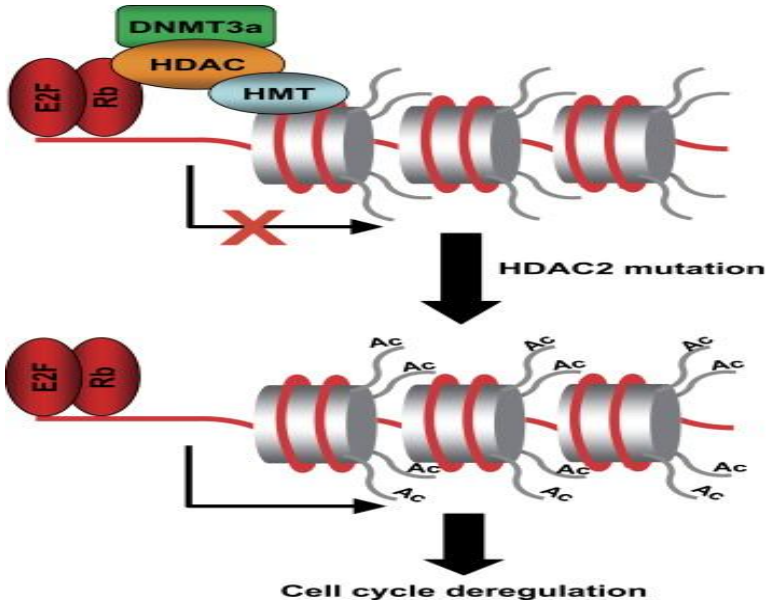

Fig.4. A model showing a possible effect of HDAC2 mutation in cancer development. Class I HDACs are involved in gene transcription-repression mediated by retinoblastoma protein. The loss of HDAC2 function could induce the hyper acetylation and reexpression of genes regulated by retinoblastoma protein Rb, and with crucial functions in cell cycle regulation. (Singh et al; 2010)

functional assays to confer resistance to the anti-proliferative and pro apoptotic effects of HDAC inhibitors (S.Ropero et al., 2006).HDAC4 mutations have been identified in breast cancer samples at significant frequency in the recent large-scale sequencing study of breast and colorectal cancers (T. Sjoblom et al., 2006)

2.4 EXPRESSION AND FUNCTION OF HDACS IN CANCER

HDAC1 expression. In a first study of leukemia cells, HDAC1 expression was up regulated in compared with normal tissue (J.H. Choi et al., 2001). Elevated class I HDAC expression was significantly associated with nodal spread and it was an independent prognostic marker for survival of patients affected with gastric cancer (W. Weichert et al., 2008). In pancreatic cancer, it was observed that high HDAC1 expression combined with HIF1were associated with low prognosis in a series of 39 pancreatic carcinomas (K. Miyake et al., 2008) Consequently, in a larger study involving 192 pancreatic carcinoma samples, high HDAC 1, 2, 3 expressions was observed in those which are associated with dedifferentiation and enhanced proliferation of

pancreatic cancer cells (W. Weichert et al., 2008). In colorectal cancer, it was observed that there was increased HDAC1 expression along with HDAC5, 7 were showed in contrast to breast, and bladder cancer(H. Ozdag et al., 2006). In a recent studies of 140 colorectal cancer samples, having high HDAC1, 2, 3 expression levels indicates reduced patient survival, with HDAC2 expression being an independent prognostic factor (W. Weichert et al., 2008). In prostate cancer samples, HDAC1 protein expression was observed maximum in hormone refractory, and high grade cancer compared with low grade cancer and ultimately with benign prostatic hyperplasia (K. Halkidou et al., 2004). In hepatocellular carcinoma, high HDAC1 expression was in association with cancer cell invasion into the portal vein, a stage of poorer histological differentiation, and a more advanced TNM stage and poor survival of patients in 47 cases (T. Rikimaru et al., 2007). In lung cancer, higher HDAC1 expression in advanced stage disease was compared with low stage, but no difference was compared with normal lung tissue was reported in a series of 102 samples studied (H. Sasaki et al., 2005). In 200 breast cancer samples, HDAC1 and 3 expression was found to associate with estrogen and progesterone receptor expression and HDAC-1 expression was predicted to be better disease free survival. Multivariate analysis demonstrated that HDAC1 was an independent prognostic marker (C.A. Krusche et al., 2005). In another studies of 162 breast cancer samples, high HDAC1 expression was associated with better survival, negative lymph node status and small tumor size (Z. Zhang et al., 2005). Taken both together, these studies show that HDAC1 overexpression appears especially common in cancers of the gastrointestinal system and is associated with dedifferentiation of cells, enhanced proliferation, invasion, advanced disease and leads poor prognosis. However, these studies mostly investigated only HDAC1 and not any other HDAC family member. Essential function of HDAC1 in proliferation control and p21 and p27 CDK inhibitor repression has been described and shown in mouse embryonic stem cells (K.B. Glaser et al., 2003). In cancer cells, several studies have found an important function of HDAC1 in controlling cell proliferation. HDAC1 and 3 inactivation resulted in inhibition of cell proliferation of HeLa cells, whereas inactivation of HDAC4 and 7 did not lead to decreased cell numbers (K.B. Glaser et al., 2003). Knockdown of HDAC1 results in arrest either at the G (1) phase of the cell cycle or at the G(2)/M transition, that causes loss of mitotic cells and hence leads to activation of oncogenes, leads to cell growth inhibition that leads to cancer stage, and an increase in the percentage of apoptotic cells in osteosarcoma and breast cancer cells (S. Senese et al., 2007). On the other way, HDAC2

knockdown showed no such effects in these cells (S. Senese et al., 2007). Short interval of interference of RNA-based inhibition of HDAC1 and HDAC2 suppresses the growth of colon cancer cells in vitro (W. Weichert et al., 2008). HDAC1 overexpression leads to an increase in proliferation and to an undifferentiated phenotype in cultured prostate cancer cells (K. Halkidou et al., 2004). In addition to controlling cell cycle and apoptosis, it has been thought that HDAC1 might also be associated with multidrug resistance. HDAC1 was overexpressed in chemotherapy resistant neuroblastoma cells that was in vitro and siRNA knock down sensitized cells for etoposide treatment (N. Keshelava et al., 2007). HDAC1 knockdown by small interference RNA stimulated urokinase plasminogen activator expression and invasion of neuroblastoma cells in vitro, which was also observed using the unselective HDAC inhibitors TSA, butyrate and scriptaid(S.M. Pulukuri et al., 2007)However, recent findings shows that the HDAC inhibitor HC toxin efficiently inhibits migration and invasion of MYCN amplified neuroblastoma cells (H.E. Deubzer et al., 2008), which could be due to the unique features of this compound compared with other HDAC inhibitors investigated in the same culture model (H.E. Deubzer et al., 2008) and (H.E. Deubzer et al ., 2008). HDAC1 targeting was recently shown in HeLa cells to induce autophagy (M. Oh et al., 2008). Knockdown of HDAC1 and HDAC2 but not HDAC3, HDAC6, and HDAC8 was sensitizes CLL cells for TRAIL-induced apoptosis (S. Inoue et al., 2006)

2.5 MOLECULAR MECHANISM OF HDAC INHIBITORS IN ANTICANCER EFFECTS

There are several enzymes, which include acetylates and deacetylases, which can regulate transcription by modifying and changing the acetylation state of histones or other promoter-bound transcription factors. These enzymes reveal their involvement in cell-cycle regulation and differentiation. Furthermore, it was suggested that deregulation of acetylase and deacetylases activity plays a causative role in the generation of cancer (Kouzarides T., 1999). Restraining HDAC activity and preventing the deacetylation of histone may induce hyper acetylation of histone that is removal of acetyl moieties, which results in unfolding of ordered chromosome and promote transcription factors combined with DNA, so genes which are inhibited can express and exert the effect of cure tumor (Brown R and Strathdee G.., 2002) (Fig.5). HDAC enzymes remove the acetyl moieties from the histone and give rise to the condition (hypo acetylation),

which in turn decrease the space between the nucleosome and the DNA wrapped around it, decreasing the transcription factor access and that leads to transcriptional repression (De Ruijter etal.,2003).

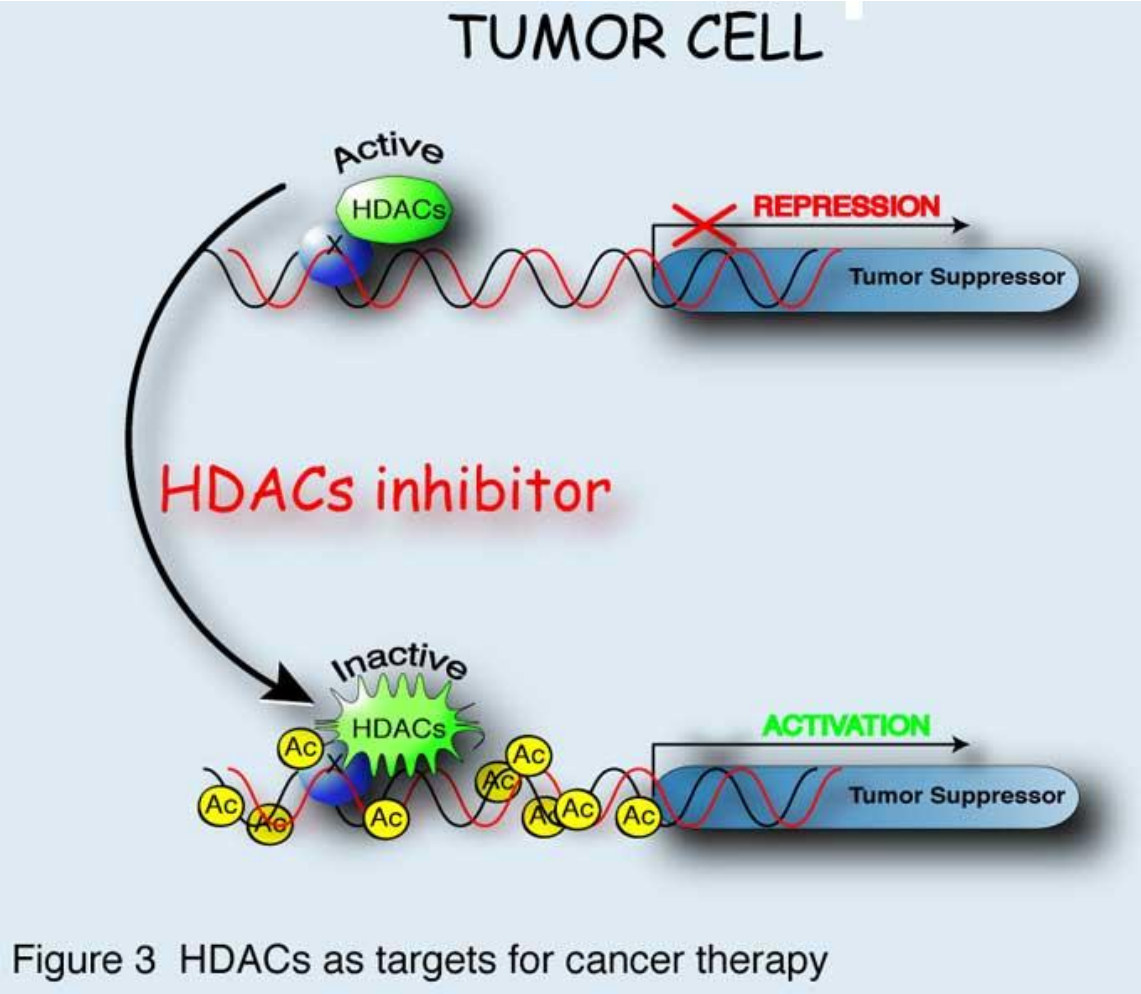

Fig.5. Mechanism of HDACs inhibition in flow chart manner (Chiocca, 2010**)**

Catalytic domain of the HDAC was formed by a stretch of 390 amino acids which contains a set of conserved residues. The active site of the enzyme is occupied by a curved tubular pocket with a wide bottom. Removal of an acetyl group occurs through a charge-relay system, an important component, to which is the zinc-binding site at the bottom of the pocket. The presence of a zinc ion at this site plays an important factor in the mechanism of action of HDAC inhibitors.

2.6 EFFECTS OF HDAC INHIBITORS ON SOLID TUMOR CELLS

In study of HT-29 cells, it was seen that sodium butyrate-mediated growth inhibition is associated with a marked decrease in cyclin B1 mRNA levels. These decreased in cyclin B1 occurred in a delayed fashion (at 24 h), and is completely blocked by concomitant treatment with protein synthesis inhibitors, and it appears to be dependent on changes in transcription. Cyclin B1 repression is likely to be linked to the differentiation process in colon cancer cells but it is observed that it is not relation with growth arrest. The mechanism which required the repression of cyclin B1 by butyrate requires prolonged histone hyper acetylation and is at least it is partly dependent on p21 expression. In fact, p21/WAF-1 appears to direct repress a minimal cyclin B1 promoter (-90 bp), a process that can be mediated by the amino-terminal portion of the p21 protein (Archer SY, et al., 2005). TSA or NaBu blocked two colon cancer cell lines (SW1116 and Colo-320) mainly in the G1 phase. In these two human colon cancer cell lines, HDAC inhibitors increased the p21 (WAF1) gene expression by selectively increasing the degree of acetylation of the gene-associated histones, and induced a G1 cell cycle arrest (Chen YX, et al., 2004). The histone deacetylases inhibitor-induced apoptosis for three pancreatic adenocarcinoma cell lines (IMIM-PC-1, IMIM-PC-2 andRWP-1) due to a serine protease-dependent and caspase independent mechanism. Histone deacetylase inhibitors increase Bax protein levels without being affecting Bcl-2 levels. Consequently, this induces the apoptosis-inducing factor (AIF) and Omi/HtrA2 are released from the mitochondria, with the subsequent starting of the apoptotic program. These mechanisms require AIF relocalization into the nuclei that induce DNA fragmentation and a serine protease activity of Omi/HtrA2 (Garcia-Morales P et al., 2005). Apoptosis of a human lung carcinoma cell line (A549) by TSA was associated with a down-regulation of anti-apoptotic Bcl-2 protein and an up-regulation of pro-apoptotic Bax protein hence Bcl-2 and BAX protein acts opposite to each other one up regulates and other one down regulates the pro apoptotic cells. TSA treatment induced the proteolytic activation of caspase-3 and caspase-9, which results in degradation of poly (ADP-ribose)-polymerase protein. Furthermore, it was observed that TSA decreased the levels of COX-2 mRNA and protein expression without significant changes at the level of COX-1so TSA brings about the changes in COX-2 and not in COX-1, which was correlated with an inhibition in prostaglandin E2 synthesis (Choi YH., 2005). Melanoma cells most of the time retain wild-type p53 tumor suppressor protein and express it at high levels which suppresses the p-53 pathway of apoptotic mechanism.

TSA can stabilize wild-type p53, but due to this p53 protein accumulation was overridden by simultaneous down regulation of p53 mRNA leading to a decrease in p53 protein. Growth arrest was induced in all cell lines studied and apoptosis in most (6/7), these cellular effects were independent of the p53 status of the cells. Dominant negative p53 (175His) inhibits p53 functions that confirms that the HDAC inhibitor induced apoptosis was independent of wild-type p53, even though TSA slightly activated p53. The results shows that p53 does not have any action on TSA ,which in turns activates the apoptosis pathway by the HDAC inhibitors that may provide therapeutic approaches for melanoma treatment (Peltonen K et al., 2005). Breast cancer cell-conditioned medium enhanced phosphorylation of activator transcription factor-2 (ATF-2) which was the main modulatory subtype in CREB/ATF-2 family. Inhibition of phosphorylation of ATF-2leads to the effect of sodium butyrate on aromatase expression in breast tumor fibroblast, and hence, the inhibition of binding of a transcriptional complex containing phosphorylated ATF-2, C/EBP β and CREB binding protein (CBP) binds to promoter II and I, 3 regulatory region. Level of aromatase mRNA arising from cancer-induced promoter region was reduced by sodium butyrate. The aberrant activation of promoter II and I,3 in HAF is dependent on ATF-2 phosphorylation (Ma D and Zhou JF, 2003). Exposing A2780 ovarian cancer cells to the histone deacetylases inhibitor TSA produced a remarkable change in cellular morphology, differentiation and proliferation. Within 24 h of TSA treatment, it was observed that there was a morphological transformation of the cells, with increased cytoplasm, a more epithelial-like columnar appearance, and the rise of distinct cellular boundaries commensurate with the morphological transformation, TSA also inhibited cell proliferation. TSA can stimulate epithelial-like differentiation with increased cytokeratin expression and the reappearance of intercellular plasma membrane junctions and primitive microvillus. In conclusion, it can be recorded that the observed TSA-induced changes in p21, Rb, and Id1 have consistent relation with cell cycle senescence and differentiation of A2780 ovarian cancer cells (Strait KA, et al., 2002).

2.7 EFFECTS OF HDAC INHIBITORS ON LEUKEMIA CELLS

Extensive research of the effect *in vitro* of many HDAC inhibitor for different tumor cell lines showed that HDAC inhibitor can lead many leukemia cell lines to different extent of differentiation, apoptosis(cell death) and can block cell circle at G0~G1 period or G2~M period

hence HDAC regulates the cell cycle and leads to leukemia conditions. The effect depends on the type of cell lines, different drugs and action time. Activated fusion proteins such as PML-RARα have been shown to inhibit cellular differentiation by recruitment of nuclear co repressor complexes, which maintain local HDAC in a variety of hematologic lineage-specific gene promoters. HDAC dependent transcriptional repression appears as a common pathway in the development of leukemia and could constitute an important target for new therapeutic agents (Martinez-Mancilla M et al., 2006). Phenyl butyric acid can induce the differentiation and apoptosis of cell lines U937, HL-60, ML-1, K562, NB4, Kasumi-1, et al. Butyric acid and TSA can induce NB4 cell line differentiation and apoptosis, and obtain same results of acute premyelocytic leukemia patient's primary cell (Melnick A and licht JD, 2002) and (Rahmani M, et al., 2003). In chronic myelocytic leukemia (CML) the activity of the Bcr-Abl tyrosine kinase is known to activate a number of molecular mechanisms, which inhibit apoptosis. SAHA induced apoptosis in BV-173 cells, which involves decreased protein expression levels of Bcr-Abl, c-Myc and HDAC3 (Xu Y,et al., 2005). Depsipeptide can up-regulate IL-3 gene expression of AML1/ETO positive leukemia cell, and IL-3 is essential signal transduction regulating gene for normal hematopoiesis (Klisovic MI, et al., 2003). Apicidin might induce apoptosis of HL-60 cell through the help of selective induction of Fas/Fas ligand hence it helps in apoptosis, resulting in the release of cytochrome c from the mitochondria to the cytosol and subsequent activation of caspase-9 and caspase-3 (Kwon SH, et al., 2002). Low dose of sodium butyrate and Trichostatin can induce K562 cell line differentiation. They can block K562 cell cycle in different stage, but the differentiation both through inducing P21 and cyclin D3 expresses (Shankar S, et al., 2005). HDAC inhibitors enhance the apoptosis-inducing potential of leukemia cells lines like (HL60, Jurkat, K562, and U937) through a series of multiple mechanisms, which can up-regulate DR4, DR5, Bak, Bax, Bim, Noxa and PUMA, down-regulate IAPs, Mcl-1, Bcl-2, Bcl-XL and cFLIP, and release mitochondrial proteins like (cytochrome c, Smac/DIABLO and Omi/Htr2) to the cytosol, induct p21WAF1/CIP1 and p27KIP1, activate caspase-3 and cleave poly (ADP-ribose) polymerase (PARP). The up regulation of death receptors and inhibition of cFLIP by HDAC inhibitors will increase the ability of TRAIL to induce apoptosis, it is due to enhance activation of caspase-8,and cleavage of Bid, which releases mitochondrial proteins to the cytosol, and in turn leads to the activation of caspase-9 and caspase-3 (Shankar S, et al., 2005). The link between altered HDAC activity and tumor genesis is probably best

demonstrated in acute promyelocytic leukemia (APL). The retinoic acid receptor (RAR) transcription factors RARa and its hetero dimerization partner RXR bind to retinoic acid response elements (RAREs) and, in the absence of retinoids, repress transcription through a complex involving SIN3/HDAC, NCOR and SMRT this complexes helps in repression of transcription. Addition of retinoic acid enables HATs (such as TIF2 and CBP) to replace the HDACs, thereby activating transcription and ultimately activating tumor repressor genes (Altucci L, Gronemeyer H.., 2001) and (Johnstone RW., 2002). HDAC inhibitor can induce many lymphocytic leukemia differentiation and apoptosis. Whereas depsipeptide induce bd-6 positive Raji cell and lymphocytic leukemia cell cycle blockage and apoptosis, chronic lymphocytic leukemia cell and myeloma cell are sensitive to HDAC inhibitor either (Rosato RR, et al., 2002). SAHA can induce diffuse large cell lymphoma and Hodgkin disease cell lines apoptosis. In contrast to that HDAC inhibitors are very sensitive to IL-2 dependent cell lines, there have extensive acetylation in these cells, but target genes (like myc) of IL-2 pathway were inhibited indicating CD25+（IL-2R）tumor can be treated by HDAC inhibitors (Aron JL etal., 2003). As been noted above, the anticancer mechanism of HDAC inhibitor is dependent on the regulation of gene express, large experiments *in vivo* and *in vitro* have confirmed that there are three kinds of anticancer mechanisms: 1) block cell cycle and promote cell differentiation, 2) induce cell apoptosis, 3) inhibit angiogenesis.

2.8 PERSPECTIVE

Anticancer and antagonistic actions of HDAC inhibitors due to inhibition in deacetylation of histone mainly thus relieve transcription inhibition of some genes. More genes whose transcription activation is related with histone acetylation should be detected following deep research of cell proliferation, differentiation and apoptosis, and translation to corresponding protein produce further effect. But histone acetylation/ deacetylation as a spot of complex gene express regulating net in eukaryotes, is influenced necessarily by other biomolecules. HDAC inhibitor has other drug action target except histone: HDAC can induce some hertones as transcription factor P53, GATA21, NF2YA deacetylation and regulating activity of binding to DNA (Richon VM and O'Brien JP.., 2002). Therefore HDAC inhibitor may selective induce some gene expression related with tumor cell differentiation and apoptosis through regulating acetylation level of these transcription factors. Careful trial design is important in order to fully exploit agents such as the HDAC inhibitors. The use of surrogate markers that does the activity

will be important, as a detailed appreciation of their mechanism of action to ensure the optimal clinical application in cancer of these agents. The fact that several HDAC inhibitors are in early stage clinical trials means we can expect to see an increasing number of published reports on their efficacy and potential clinical application. Only understanding common, concrete, key and special molecular mechanism of certain kind of tumor invasion, we can choose HDAC inhibitor for therapy especially for anticancer drugs. In this way, HDAC inhibitor may become another successful inducing differentiation agent for some kinds of tumor after all-trans retinoid acid treat acute pre myelocyticleukemia.

3. OBJECTIVE

In the light of above text two objectives are decided to fulfill during M.Sc project

- To compare the RNA isolation from manual method and by kit method
- To determine the expression profile of histone deacetylases (HDAC) 1 in leukemia cells and normal cells

4. MATERIALS AND METHODS

4.1 COLLECTION OF SAMPLES:

Blood was collected as the normal tissue from the local CWS Hospital, Rourkela, Odisha, stored in ice and immediately processed for better RNA extraction. Cancer tissue (Lymph Node Carcinoma) was collected from National Medical College, Kolkata and stored in 4^{o}C until RNA was extracted.

4.2 EXTRACTION OF TOTAL RNA:

Total RNA was extracted from blood (normal) and cancer tissue using GeneJET^{TM}RNA Purification Kit (Fermentas), Manual RNA Extraction techniques and Trizol method.

4.2.1 EXTRACTION FROM BLOOD BY RNA PURIFICATION KIT:-

The collected blood was centrifuged at 3000 rpm for 15 mins at 4° C. The supernatant containing the serum was separated from the pellet which contains the blood cells. The pellet was re suspended in 600 µl of Lysis Buffer (which is supplemented with 20 µl of 14.3, M β-mercaptoethanol for every 1ml of Lysis Buffer) and that was vortexed so that it will mix thoroughly. 450 µl of ethanol (96-100%) was mixed with the solution. About 700 µl of the lysate was transferred to a GeneJET^{TM}RNA Purification Column inserted in a collection tube and centrifuged at 12000 rpm for 1 min at 4° C. The flow-through was discarded and the column was placed into a new 2 ml RNase-free micro centrifuge tube. 700 µl of that Wash Buffer 1(supplemented with 250 µl of ethanol for every 1ml Wash buffer 1) was added to the given column and centrifuged for 1 min at 12000 rpm. The flow-through was discarded and 600 µl of Wash Buffer 2 (supplemented with 850 µl of ethanol for every 0.5 µl Wash buffer 2) was added to the column. It was centrifuged at 12000 rpm for 1 min at 4° C. The flow-through was again discarded. Centrifugation was again done at 12000 rpm for 1 min at 4 ° C by adding 250 µl of Wash buffer 2. The flow-through was discarded and the column was transferred to a sterile 1.5 RNase-free microcentrifuge tube which is essential. 100 µl of nuclease-free water was added to the column and centrifuged for 1 min at 12000 rpm to elute RNA. The RNA was stored at - 20° C for further use or immediately processed for cDNA synthesis.

4.2.2. mRNA EXTRACTION FRON BLOOD BY TRIZOL RNA EXTRACTION TECHNIQUES:-

Chemical Reagents and Buffer used during practical-chemical reagents (TRIzol reagents (sigma), chloroform, isoproponol, ethanol (70%). Denaturation buffer consists of 50% deionized formamides that consists of (2.2 M formaldehyde, MOPS buffer (P^H -7.0), 6.6% glycerol, 0.5% bromophenol, ethidium bromide and agarose.

PROTOCOL-

50-500 mg of frozen tissue was transferred to a 2ml tube with 1ml TRIzol. It was homogenized for 60 sec in the polytron. 200µl of chloroform was added to it. It was mixed by inverting the tube for 15 sec then incubated for 3 min at room temperature then it was centrifuged at 12.000g for 15 min. aqueous phase was transferred into a fresh Eppi tube. To it added 50µl of isopropanol. It was centrifuged at max 7500g for 5 min in the cold room. Pellet was dried on air for 10 min it was dissolved in 50-100µl DEPC-H_2O and incubated for 10 min at 60^oc. After that spectrophotometer reading was taken. The RNA on a MOPS gel was analysed by the following process- 1-3µg RNA was dissolved in 11µl of denaturation buffer, 1µl of ethidium bromide (1mg/ml) was added and denatured at 65^oc for 15 min. it was loaded at 1% agarose gel in MOPS buffer plus 5% formaldehyde. Gel was run at 40v for 4 hours

4.2.3. mRNA EXTRACTION BY MANUAL METHOD:-

Manually mRNA was extracted by using chemical reagents and buffer

Chemical Reagents and Buffer:-

a) Chloroform isoamyl alcohol - 49:1
b) Ethanol
c) Isopropanol
d) PBS
e) Sodium Acetate
f) Solution D(Denaturating Solutions)

Guanidium Thiocyanate

Sodium Citrate

Sodium Lawryl Sarcosinate

β Mercaptoethanol

PROCEDURE:-

The collected blood sample was centrifuged at 3000rpm for 10 min (room temperature). PBS was added and centrifuged (room temperature).The pellet was collected and 2ml of solution D was added for 106 cells. The cells were homogenized for 15 to 30 sec in room temperature. 0.1ml of 2M sodium acetate (pH-4.0), 1ml of phenol and 0.2ml of chloroform: isoamyl alcohol per milliliter of solution D was added. It was mixed thoroughly by inversion. The homogenate was gently vortexed vigorously for 10 sec; the tube was incubated for 15 minutes on ice to permit complete dissociation of nucleoprotein complex. The tube was centrifuged at 9000rpm for 20 min at 4°C. The extracted RNA was collected in the upper aqueous solution. An equal volume of isopropanol was added to the extracted RNA and the solution was mixed and the RNA was allowed to precipitate for 1min at -20°C. RNA was collected by centrifugation at 9000rpm at 4°C for 30 min. Isopropanol was carefully decanted and the RNA pellet was dissolved in 0.3ml Solution D for every 1ml of solution used in early step. The solution was transferred to a microfuge tube, it was vortexed well and the RNA was precipitated with 1 volume of isopropanol for1 hour or more at -20°C. The precipitate RNA was collected by centrifuge at maximum speed for 10mins at 4°C.The pellet was washed twice with 75% ethanol. It was again centrifuged and allowed to dry completely.

4.2.4 For Extraction from Cancer tissue:

About 30 mg of frozen cancer tissue was taken and thoroughly homogenized using Lysis buffer. The homogenized tissue was transferred into a sterile 2 ml micro centrifuge tube containing 300 µl of Lysis Buffer (supplemented with 20 µl of 14.3 M β-mercaptoethanol/1ml of Lysis Buffer). The mixture was thoroughly mixed by vortexing for 10 sec. The next steps of extraction were same as that followed in the previous protocol for blood RNA extraction

4.3 QUANTITATIVE ESTIMATION OF RNA CONCENTRATION BY SPECTROPHOTOMETRIC ANALYSIS:

The concentration of the extracted total RNA from both blood and cancer tissue was quantified by measuring the absorbance at 260 nm in a spectrophotometer (ELICO, BL 200 Bio Spectrophotometer, double beam) and calculated by using the formula as given below (Table 1)

$$\text{Total RNA } (\mu g /ml) = OD_{260} \times 40 \times \text{Dilution factor.}$$

Tissue	Concentration (µg/ml)	Purity (260/280) nm	Purity(260/230) nm
Normal tissue (Blood)	570.32	1.34	0.82
Cancer (leukemia cells)	234.67	1.03	0.65

Table 1. Concentration and purity measurement of leukemia cells and normal cells by spectrophotomeric method.

4.4 QUANTITATIVE ESTIMATION OF RNA CONCENTRATION BY DENATURING GEL ELECTROPHORESIS:

The extracted RNA from both blood and cancer tissue was run on a denaturing agarose gel and the quantity of RNA estimated from the band intensity. For denaturation gel (40 ml), 0.6 g agarose (Sigma), 28.8 ml dH_2O (Sigma), 7.2 ml formaldehyde (Sigma), 4 ml 10X MOPS buffer were mixed properly. About 2 µl (2µg) of the total RNA was mixed with 18 µl 1X Reaction Buffer (2µl of 10X MOPS Buffer, 4 µl formaldehyde, 10 µl formamide (Sigma) ,2 µl 0.2 mg/ml Etbr (Sigma)) and incubated at 55 °C for 1 hr. It was then cooled on ice and loaded in the wells of the denaturing gel.

4.5. FIRST STRAND cDNA SYNTHESIS:

Total RNA (4 µg) from both blood and cancer tissue were used for first strand cDNA synthesis by reverse transcription using RevertAid^{TM}First Strand cDNA Synthesis Kit (Fermentas) in a

thermo cycler(Bio-Rad). The RNA were incubated with 1 µl of oligo (dT)primers(100 µM, 0.2 µg/µl) and 12 µl of nuclease-free water at 65°C for 5 min.The reaction was cooled on ice to allow the primers to anneal to the RNA, then spin down and placed on ice again after which the following components were added to the reaction in order; 4µl of 5X Reaction Buffer, 1 µl of Ribolock^{TM}RNase inhibitor (20 U/µl), 2 µl of 10mMdNTPsand 1.0 µL of RevertAid^{TM} M-MuLV-Reverse Transcriptase (200 U/µl). The reagents were gently mixed and were incubated for 1 h at 42°C which is ambient temperature used for the practical. Heating at 70°C for 5 min terminated the reaction and the synthesized cDNA was stored at –20 ^{0} C for further use.

4.6. GENE-SPECIFIC PCR FOR AMPLIFICATION OF THE DESIRED GENE:-

4.6.1 SELECTION OF PRIMERS:

A set of specific forward and reverse primers for the amplification of the desired gene under study was selected from a set of published papers (Patra et al., 2003; Zou et al., 2002) and primer selection was done. The cDNA of both the blood and cancer tissue synthesized were used as the template for the specific primers. The constitutively expressed housekeeping gene, β-actin was used as a positive control to ensure high quality. The primer sequences used for the PCR reaction are shown in Table showing the sequence of the forward and backward primers.

GENE	PRIMERS
HDAC1	FORWARD- 5'ACGGTCTTTATAAGAAGATGATCGT-3'
	REVERSE- 5'CTGGGAGGCCTGGTACGGCTTGAAG-3'
β-ACTIN	FORWARD-5' TCTACAATGAGCTGCGTGTG 3'
	REVERSE-5' TCTCCTTCTGCATCCTGTC 3'

4.6.2 PCR CONDITIONS:

The PCR sample mixtures, in a 25 µl volume, contained 17 µl of dH_2O (Sigma), 2.5 µl of 1XPCR buffer (Sigma), 0.5 µl of dNTP (0.2mM, Sigma), 1.5 µl of $MgCl_2$ (1.5 mM, Sigma), 0.5 µl each of the forward and reverse primers (0.2 µM, Sigma) of MBD1, MBD2, MBD3, MBD4 and 0.5 µlTaq DNA-polymerase (1U/µl, Himedia). 2 µl of each cDNA sample was added. PCR amplifications of MBD1, MBD2, MBD3 and MBD4 were performed in a thermal cycler by initial denaturation at 94° C for 1 min, followed by 30 cycles of denaturation at 94° C for 20 secs, annealing at 57° C for 20secs, and extension at 72° C for 30 secs, followed by an final extension step at 72° C for 5 mins. For amplification of cDNA , the following conditions were followed:initial denaturation at 95° C for 5 mins, prior to 30 cycles of denaturation at 94° C for 30 secs, annealing at 57° C for 20secs, and extension at 72° C for 45secs, followed by an final extension step at 72° C for 10mins.

4.7 AGAROSE GEL ELECTROPHORESIS OF THE PCR PRODUCTS:-

The generated PCR products were analyzed by electrophoresis on 1.5% agarose gel. Agarose gel was prepared with 1X TAE (Tris Acetate EDTA, Sigma) buffer. Before casting 0.1% ethidium bromide was added to the gel. 15 µl of sample (PCR product) was loaded to each well along with 3 µl 1 X loading dye. 5 µl of DNA marker (1 kb, Sigma). The gel was run in TAE buffer at 100 volt for 40 minutes

4.8 ANALYSIS OF THE RELATIVE EXPRESSION LEVEL OF THE DIFFERENT GENES:

The relative levels of expression of each gene were analyzed by taking the absorbance through spectrophotometric readings. The ratios of desired genes/β-actin product were subsequently calculated after subtraction of the background pixel intensity for each gene of interest and used to assess the differences in expression levels between normal and cancer tissue.

5. RESULT AND DISCUSSION

In study of leukemia cells HDAC-1 expression was up regulated compared with the normal cells. The observation was confirmed by doing experiment in lab. Experiment was performed by RNA isolation from both normal cells and leukemia cells and its expression was checked by running agarose gel. Beta actin a house keeping gene that shows same expression in all kind of cells was visualized for its expression. When normal cell was compared with leukemia cells HDAC-1 expression was nil in normal cells while well bright band was observed in leukemia cells.

RNA isolation was done by two methods one bye kit and other by manually and hence it was observed that RNA isolation was maximum and pure when it was extracted manually rather then by kit.

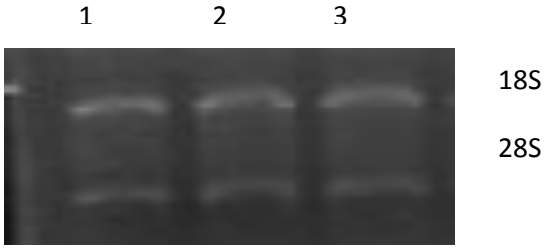

Fig.6 denaturing gel for normal cells

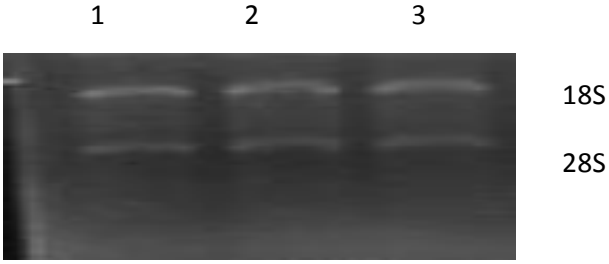

Fig.7 denaturing gel for leukemia cells

Fig.8 HDAC-1 expression in normal cells

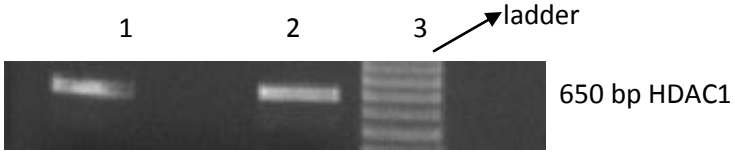

Fig. 9 HDAC-1 expression in leukemia cells

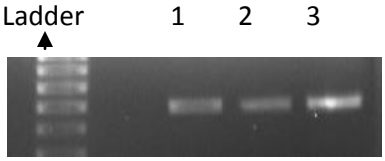

Fig.10 beta actin expression normal cell

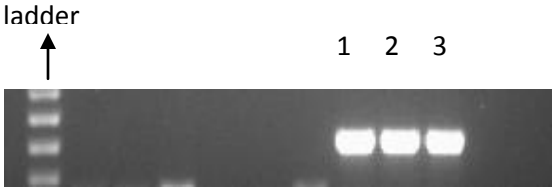

Fig.11 beta actin expression in leukemia cells

6. CONCLUSION

Acetylation and Deacetylation is two regulatory and exactly opposing processes in modulation of histones. Acetylation is a process of addition of acetyl group to the histone while deacetylation removes the acetyl group from the histones. From the previous experiment it was concluded that HDAC1 expression was more in cancer cells and tissues and hence it was correlated with suppression of tumor repressor and cell cycle regulatory genes. From the experiments carried out in our lab it was observed that HDAC1 expression was high in leukemia cells in compared to the normal cells. In normal cells the expression was nil. In this context it was also observed that while carrying out the experiment for HDAC1 expression RNA extraction was done by two methods one by manually and other by the help of RNA extraction kit and it was observed that RNA extraction was maximum and pure when it was extracted by manually rather than by kit. Hence, from the above experiment it was concluded that HDAC-1 higher expression suppresses the tumor repressor gene and accordingly associated with some mechanisms leading to the cancerous stage.

7. REFRENCES

A.J.Ruijter, A.H.Gennip, H.N.Caron, S. Kemp, A.B Kuilenburg., Histone deacetylases (HDACs): characterization of the classical HDAC family, Biochem. J. 370 (2003) 737–749.

Allfrey VG, Faulkner R, Mirsky AE. Acetylation and methylation of histones andtheir possible role in the regulation of RNA synthesis. Proc Natl Acad Sci USA1964;51(May):786–94.

Altucci L, Gronemeyer H. The promise of retinoids to fight against cancer. Nat Rev Cancer. 2001;1:181-193.

Archer SY, Johnson J, Kim HJ, et al. The histone deacetylase inhibitor butyrate downregulates cyclin B1 gene expression *via* a p21/WAF-1-dependent mechanism in human colon cancer cells. Am J Physiol Gastrointest Liver Physiol. 2005;289:696- 703.

Aron JL, Parthun MR, Marcucci G, et al. Depsipeptide (FR901228) induces histone acetylation and inhibition of histone deacetylase in chronic lymphocytic leukemia cells concurrent with activation of caspase-8-mediated apoptosis and down regulation of c-FLIP protein. Blood. 2003;102:652-658.

B.L. Park, Y.J. Kim, H.S. Cheong, S.O. Lee, C.S. Han, J.H. Yoon, J.H. Park, H.S. Chang, C.S. Park, H.S. Lee, H.D. Shin, HDAC10 promoter polymorphism associated with development of HCC among chronic HBV patients, Biochem. Biophys. Res. Commun. 363 (2007) 776–781.

Biola M. Javierre, Manel Esteller, Esteban Ballestar Epigenetic connections between autoimmune disorders and haematological malignancies (2008).

Brahma N Singh, Guanghua Zhang, Yi L Hwa, Jinping Li, Sean C Dowdy and Shi-Wen Jiang Nonhistone protein acetylation as cancer therapy targetsJ une 2010, Vol. 10, No. 6, Pages 935-954

Brown R, Strathdee G. Epigenomics and epigenetic therapy of cancer. Trends Mol Med. 2002;8:43-48

C.A. Krusche, P. Wulfing, C. Kersting, A. Vloet, W. Bocker, L. Kiesel, H.M. Beier, J. Alfer, Histone deacetylase-1 and -3 protein expression in human breast cancer: a tissue microarray analysis, Breast Cancer Res. Treat. 90 (2005) 15–23.

Cebrian, P.D. Pharoah, S. Ahmed, S. Ropero, M.F. Fraga, P.L. Smith, D. Conroy, R. Luben, B. Perkins, D.F. Easton, A.M. Dunning, M. Esteller, et al, Genetic variants in epigenetic genes and breast cancer risk, Carcinogenesis 27 (2006) 1661–1669.

Chen YX, Fang JY, Lu J, et al. Regulation of histone acetylation on the expression of cell cycle-associated genes in human colo cancer cell lines. Zhonghua Yi Xue Za Zhi. 2004;84:312-317.

Choi YH. Induction of apoptosis by trichostatin A, a histone deacetylase inhibitor, is associated with inhibition of cyclooxygenase-2 activity in human non-small cell lung cancer cells. Int J Oncol. 2005;27:473-479.

Cress WD, Seto E. Histone deacetylases, transcriptional control, and cancer. J Cell Physiol 2000;184(July (1)):1–16.

de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histonedeacetylases (HDACs): characterization of the classical HDAC family. Biochem J 2003;370(March (Pt 3)):737–49.

De Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003;370:737-749.

E. Verdin, F. Dequiedt, H.G. Kasler, Class II histone deacetylases: versatile regulators, Trends Genet. 19 (2003) 286–293.

Garcia-Morales P, Gomez-Martinez A, Carrato A, et al. Histone deacetylase inhibitors induced caspase-independent apoptosis in human pancreatic adenocarcinoma cell lines. Mol Cancer Ther. 2005;4:1222-1230.

Glaser KB, Li J, Staver MJ, Wei RQ, Albert DH, Davidsen SK. Role of class I and class II histone deacetylases in carcinoma cells using siRNA. Biochem Biophys Res Commun 2003;310:529–36.

Glozak MA, Sengupta N, Zhang X, Seto E. Acetylation and deacetylation of nonhistone proteins. Gene 2005;363(December):15–23.

Grozinger CM, Schreiber SL. Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. Chem Biol 2002;9:3–16.

H. Ozdag, A.E. Teschendorff, A.A. Ahmed, S.J. Hyland, C. Blenkiron, L. Bobrow, A. Veerakumarasivam, G. Burtt, T. Subkhankulova, M.J. Arends, V.P. Collins, D. Bowtell, et

al, Differential expression of selected histone modifier genes in human solid cancers, BMC Genomics 7 (2006) 90.

H. Sasaki, S. Moriyama, Y. Nakashima, Y. Kobayashi, M. Kiriyama, I.Fukai, Y. Yamakawa, Y. Fujii, Histone deacetylase 1 Mrna expression in lung cancer, Lung Cancer 46 (2004) 171–178.

H.E. Deubzer, V. Ehemann, A.E. Kulozik, F. Westermann, L. Savelyeva, A. Kopp-Schneider, D. Riester, M. Schwab, O. Witt, Anti-neuroblastoma activity of Helminthosporium carbonum (HC)- toxin is superior to that of other differentiating compounds in vitro, Cancer Lett. (2008).

H.E. Deubzer, V. Ehemann, F. Westermann, R. Heinrich, G. Mechtersheimer, A.E. Kulozik, M. Schwab, O. Witt, Histone deacetylase inhibitor Helminthosporium carbonum (HC)-toxin

Hassig CA, Fleischer TC, Billin AN, Schreiber SL, Ayer DE. Histone deacetylase activity is required for full transcriptional repression by mSin3A. Cell 1997; 89:341–7.

Huang BH, Laban M, Leung CH, et al. Inhibition of histone deacetylase 2 increases apoptosis and p21Cip1/ WAF1 expression, independent of histone deacetylase 1. Cell Death Differ 2005;12:395–404.

Hubbert, A. Guardiola, R. Shao, Y. Kawaguchi, A. Ito, A. Nixon, M. Yoshida, X.F. Wang, T.P. Yao, HDAC6 is a microtubule-associated deacetylase, Nature 417 (2002) 455–458.

Humphrey GW, Wang Y, Russanova VR, et al. Stable histone deacetylase complexes distinguished by the presence of SANT domain proteins CoREST/kiaa0071 and Mta-L1. J Biol Chem 2001;276:6817–24.

I.V. Gregoretti, Y.M. Lee, H.V. Goodson, Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis, J. Mol. Biol. 338 (2004) 17–31.

J. Ahringer, NuRD and SIN3 histone deacetylase complexes in development, Trends Genet. 16 (2000) 351–356.

J. Luo, F. Su, D. Chen, A. Shiloh, W. Gu, Deacetylation of p53 modulates its effect on cell growth and apoptosis, Nature 408 (2000) 377–381.

J.H. Choi, H.J. Kwon, B.I. Yoon, J.H. Kim, S.U. Han, H.J. Joo, D.Y. Kim, Expression profile of histone deacetylase 1 in gastric cancer tissues, Jpn. J. Cancer Res. 92 (2001) 1300–1304.

J.J. Kovacs, P.J. Murphy, S. Gaillard, X. Zhao, J.T. Wu, C.V. Nicchitta, M. Yoshida, D.O. Toft, W.B. Pratt, T.P. Yao, HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor, Mol. Cell 18 (2005) 601–607.

J.M. Park, G.Y. Lee, J.E. Choi, H.G. Kang, J.S. Jang, S.I. Cha, E.B. Lee, S.G. Kim, C.H. Kim, W.K. Lee, S. Kam, D.S. Kim, et al, No association between polymorphisms in the histone deacetylase genes and the risk of lung cancer, Cancer Epidemiol. Biomarkers Prev. 14 (2005) 1841–1843.

Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. Nat Rev Drug Discov. 2002;1:287-299.

Juan LJ, Shia WJ, Chen MH, et al. Histone deacetylases specifically down-regulate p53-dependent gene activation. J Biol Chem 2000;27:20436–43.

K. Halkidou, L. Gaughan, S. Cook, H.Y. Leung, D.E. Neal, C.N. Robson, Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer, Prostate 59 (2004) 177–189.

K. Miyake, T. Yoshizumi, S. Imura, K. Sugimoto, E. Batmunkh, H. Kanemura, Y. Morine, M. Shimada, Expression of hypoxia-inducible factor-1a, histone deacetylase 1, and metastasis-associated protein 1 in pancreatic carcinoma: correlation with poor prognosis with possible regulation, Pancreas 36 (2008)

K.B. Glaser, J. Li, M.J. Staver, R.Q. Wei, D.H. Albert, S.K. Davidsen, Role of class I and class II histone deacetylases in carcinoma cells using siRNA, Biochem. Biophys. Res. Commun. 310 (2003) 529–536. [53]

Klisovic MI, Maghraby EA, Parthun MR, et al. Depsipeptide (FR901228) promotes histone acetylation, gene transcription, apoptosis and its activity is enhanced by DNA methyltransferase inhibitors in AML-ETO-positive leukemic cells. Leukemia.2003;17:350-358.

Kouzarides T. Histone acetylases and deacetylases in cell proliferation. Curr Opin Genet Dev. 1999;9:40-48.

Kwon SH, Ahn Sh, Kim YK, et al. Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells. J Biol Chem. 2002;277:2073-2080.

Lagger G, Doetzlhofer A, Schuettengruber B, et al. The tumor suppressor p53 and histone deacetylase 1 are antagonistic regulators of the cyclin-dependent kinase inhibitor p21/WAF1/CIP1 gene. Mol Cell Biol 2003;8: 2669–79.

Lagger G, O'Carroll D, Rembold M, et al. Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. EMBO J 2002;21:2672–81.

Li CR, Liu WL, Zhou JF, et al. The reseach and contrast of the mechanism which sodium butyrate and trichostatin promote K562 cell differentiation. Zhongguo Yao Li Xue Tong Bao.2004;20:41-45.

M. Dokmanovic, C. Clarke, P.A. Marks, Histone deacetylase inhibitors: overview and perspectives, Mol. Cancer Res. 5 (2007) 981–989.

M. Oh, I.K. Choi, H.J. Kwon, Inhibition of histone deacetylase1 induces autophagy, Biochem. Biophys. Res. Commun. 369 (2008) 1179–1183.

M.A. Kim, H.J. Kim, A.L. Brown, M.Y. Lee, Y.S. Bae, J.I. Park, J.Y. Kwak, J.H. Chung, J. Yun, Identification of novel substrates for human checkpoint kinase Chk1 and Chk2 through genome-wide screening using a consensus Chk phosphorylation motif, Exp. Mol. Med. 39 (2007) 205–212.

Ma D, Zhou JF. Molecular mechanism of inhibition of sodium butyrate on activation of tumor associated aromatase promoters. Ai Zheng. 2003;22:915.

Mahlknecht U, Hoelzer D. Histone acetylation modifiers in the pathogenesis of malignant disease. Mol Med 2000;6(August (8)):623–44.

Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylasesand cancer: causes and therapies. Nat Rev Cancer 2001;1(December(3)):194–202.

Marks PA, Breslow R. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotech 2007;25:84–90.

Martinez-Mancilla M, Zafra-de la Rosa G, Reynoso-Gomez E,et al. Molecular hemato-oncology and new specific treatment strategies for leukemia. Gac Med Mex. 2006;142:145-150.

Melnick A, licht JD. Histone deacetylases as therapeutic targets in hematologic malignancies. Curr Opin Hemat. 2002;9:322- 332.

N. Keshelava, E. Davicioni, Z. Wan, L. Ji, R. Sposto, T.J. Triche, C.P. Reynolds, Histone deacetylase 1 gene expression and sensitization of multidrug-resistant neuroblastoma cell lines to cytotoxic agents by depsipeptide, J. Natl. Cancer Inst. 99 (2007) 1107–1119.

Nakagawa M, Oda Y, Eguchi T, et al. Expression profile of class I histone deacetylases in human cancer tissues. Oncol Rep 2007;18:769–74.

Nolan L, Johnson PW, Ganesan A, Packham G, Crabb SJ. Will histone deacetylase inhibitors require combination with other agents to fulfil their therapeutic potential? Br J Cancer 2008;5:689–94.

Patra SK, Patra A, Dahiya R (2001) Histone deacetylase and DNA methyltransferase in human prostate cancer. Biochem Biophys Res Commun 287:705–713

Patra, S. K., Patra, A., & Dahiya, R. (2001). Histone deacetylase and DNA methyltransferase in human prostate cancer. Biochemical and Biophysical Research Communications, 287, 705–713.

Patra, S. K., Patra, A., Zhao, H., & Dahiya, R. (2002). DNA methyltransferase and demethylase in human prostate cancer. Molecular Carcinogenesis, 33, 163–171.

Patra, S. K., Patra, A., Zhao, H., Carroll, P., & Dahiya, R. (2003). Methyl-CpG-DNA binding proteins in human prostate cancer: expression of CXXC sequence containing MBD1 and repression of MBD2 and MeCP2. Biochemical and Biophysical Research Communications, 302, 759–766.

Peltonen K, Kiviharju TM, Jarvinen PM, Ra R, Laiho M. Melanoma cell lines are susceptible to histone deacetylase inhibitor TSA provoked cell cycle arrest and apoptosis. Pigment Cell Res. 2005;18:196-202.

R. Berdeaux, N. Goebel, L. Banaszynski, H. Takemori, T. Wandless, G.D. Shelton, M. Montminy, SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes, Nat. Med. 13 (2007) 597– 603.

R.B. Vega, B.C. Harrison, E. Meadows, C.R. Roberts, P.J. Papst, E.N. Olson, T.A. McKinsey, Protein kinases C and D mediate agonistdependent cardiac hypertrophy through nuclear export of histone deacetylase 5, Mol. Cell. Biol. 24 (2004) 8374–8385.

Rahmani M, Yu C, Reese E, et al. Inhibition of PI-3 kinase sensitizes human leukemic cells to histone deacetylase inhibitor-mediated apoptosis through p44/42 MAP kinase inactivation

and abrogation of p21 (CIP1/WAF1) induction rather than AKT inhibition. Oncogene. 2003;22:6231-6242.

Richon VM, O'Brien JP. Histone deacetylase inhibitors: A new class of potential therapeutic agents for cancer treatment. Clincal Cancer Res. 2002;8:662-664.

Robson CN. Up-regulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. Prostate 2004;59:177–89.

Rosato RR, Almenara JA, Cartee L, Betts V, Chellappan SP, Grant S. The cydin-dependent kinase inhibitor flavopiridol cisrupts sodium butyrate-induced p21WAF1/CIP1 expression and maturation while reciprocally potentiating apoptosis in human leukemia cells. Mol Cancer Ther. 2002;1:252-266.

S. Chang, S. Bezprozvannaya, S. Li, E.N. Olson, An expression screen reveals modulators of class II histone deacetylase phosphorylation, Proc. Natl. Acad. Sci. USA 102 (2005) 8120–8125.

S. Inoue, A. Mai, M.J. Dyer, G.M. Cohen, Inhibition of histone deacetylase class I but not class II is critical for the sensitization of leukemic cells to tumor necrosis factor-related apoptosis inducing ligand-induced apoptosis, Cancer Res. 66 (2006) 6785– 6792.

S. Kwon, Y. Zhang, P. Matthias, The deacetylase HDAC6 is a novel critical component of stress granules involved in the stress response, Genes Dev. 21 (2007) 3381–3394.

S. Ropero, M.F. Fraga, E. Ballestar, R. Hamelin, H. Yamamoto, M. Boix-Chornet, R. Caballero, M. Alaminos, F. Setien, M.F. Paz, M. Herranz, J. Palacios, et al, A truncating mutation of HDAC2 in human cancers confers resistance to histone deacetylase inhibition,Nat. Genet. 38 (2006) 566–569.

S. Senese, K. Zaragoza, S. Minardi, I. Muradore, S. Ronzoni, A. Passafaro, L. Bernard, G.F. Draetta, M. Alcalay, C. Seiser, S. Chiocca, Role for histone deacetylase 1 in human tumor cell proliferation, Mol. Cell. Biol. 27 (2007) 4784–4795.

S.M. Pulukuri, B. Gorantla, J.S. Rao, Inhibition of histone deacetylase activity promotes invasion of human cancer cells through activation of urokinase plasminogen activator, J. Biol. Chem. 282 (2007) 35594–35603

Shankar S, Singh TR, Fandy TE, Luetrakul T, Ross DD, Srivastava RK. Interactive effects of histone deacetylase inhibitors and TRAIL on apoptosis in human leukemia cells:

involvement of both death receptor and mitochondrial pathways. Int J Mol Med. 2005;16:1125-1138.

Song J, Noh JH, Lee JH, et al. Increased expression of histone deacetylase 2 is found in human gastric cancer. APMIS 2005;113:264–8.

Strait KA, Dabbas B, Hammond EH, Warnick CT, Iistrup SJ, Ford CD. Cell cycle blockade and differentiation of ovarian cancer cells by the histone deacetylase inhibitor trichostatin are associated with changes in P21, Rb, and Id proteins. Mol Cancer Ther. 2002;1:1181-1190.

suppresses the malignant phenotype of neuroblastoma cells, Int. J. Cancer 122 (2008) 1891–1900.

Susanna Chiocca Viral control of cellular pathways and biology of tumorigenesis, 2010

T. Rikimaru, A. Taketomi, Y. Yamashita, K. Shirabe, T. Hamatsu, M. Shimada, Y. Maehara, Clinical significance of histone deacetylase 1 expression in patients with hepatocellular carcinoma, Oncology 72 (2007) 69–74.

T. Sjoblom, S. Jones, L.D. Wood, D.W. Parsons, J. Lin, T.D. Barber, D. Mandelker, R.J. Leary, J. Ptak, N. Silliman, S. Szabo, P. Buckhaults, et al, The consensus coding sequences of human breast and colorectal cancers, Science 314 (2006) 268–274.

T.A. McKinsey, C.L. Zhang, J. Lu, E.N. Olson, Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation, Nature 408 (2000) 106–111.

Taunton J, Hassig CA, Schreiber SL. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science 1996;272(April (5260)):408–11.

Tianle Yang[1] and Anthony A. Sauve NAD Metabolism and Sirtuins: Metabolic Regulation of Protein Deacetylation in Stress and Toxicity(*June 27, 2006;)*

Timmermann S, Lehrmann H, Polesskaya A, Harel-Bellan A. Histone acetylation and disease. Cell Mol Life Sci 2001;58(May (5–6)):728–36

Tong JK, Hassig CA, Schnitzler GR, Kingston RE, Schreiber SL. Chromatin deacetylation by an ATPdependent nucleosome remodelling complex. Nature 1998;395:917–21.

U.B. Pandey, Z. Nie, Y. Batlevi, B.A. McCray, G.P. Ritson, N.B. Nedelsky, S.L. Schwartz, N.A. DiProspero, M.A. Knight, O. Schuldiner, R. Padmanabhan, M. Hild, et al, HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS, Nature 447 (2007) 859–863.

W. Gu, R.G. Roeder, Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain, Cell 90 (1997) 595–606.

W. Weichert, A. Roske, S. Niesporek, A. Noske, A.C. Buckendahl, M. Dietel, V. Gekeler, M. Boehm, T. Beckers, C. Denkert, Class I histone deacetylase expression has independent prognostic impact in human colorectal cancer: specific role of class I histone deacetylases in vitro and in vivo, Clin. Cancer Res. 14 (2008) 1669–1677.

W. Weichert, A. Roske, V. Gekeler, T. Beckers, C. Stephan, K. Jung, F.R. Fritzsche, S. Niesporek, C. Denkert, M. Dietel, G. Kristiansen, Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy, Br. J. Cancer 98 (2008) 604–610.

W. Weichert, A. Roske, V. Gekeler, T. Beckers, M.P. Ebert, M. Pross, M. Dietel, C. Denkert, C. Rocken, Association of patterns of class I histone deacetylase expression with patient prognosis in gastric cancer: a retrospective analysis, Lancet Oncol. 9 (2008) 139–148.

Wilson AJ, Byun DS, Popova N, et al. Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. J Biol Chem 2006;281:13548–58.

X.J. Yang, E. Seto, The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men, Nat. Rev. Mol. Cell Biol. 9 (2008) 206–218.

Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of

Xu Y, Voelter-Mahlknecht S, Mahlknecht U. The histone deacetylase inhibitor suberoylanilide hydroxamic acid downregulates expression levels of Bcr-abl, c-Myc and HDAC3 in chronic myeloid leukemia cell lines. Int J Mol Med. 2005;15: 169-172.

Y. Huang, S.J. Myers, R. Dingledine, Transcriptional repression by REST: recruitment of Sin3A and histone deacetylase to neuronal genes, Nat. Neurosci. 2 (1999) 867–872.

Y. Kawaguchi, J.J. Kovacs, A. McLaurin, J.M. Vance, A. Ito, T.P. Yao, The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress, Cell 115 (2003) 727–738.

Y. Zhang, S. Kwon, T. Yamaguchi, F. Cubizolles, S. Rousseaux, M. Kneissel, C. Cao, N. Li, H.L. Cheng, K. Chua, D. Lombard, A. Mizeracki, et al, Mice lacking histone deacetylase 6

have hyperacetylated tubulin but are viable and develop normally, Mol. Cell. Biol. 28 (2008) 1688–1701.

Y.D. Wen, V. Perissi, L.M. Staszewski, W.M. Yang, A. Krones, C.K. Glass, M.G. Rosenfeld, E. Seto, The histone deacetylase-3 complex contains nuclear receptor corepressors, Proc. Natl. Acad. Sci. USA 97 (2000) 7202–7207.

Z. Zhang, H. Yamashita, T. Toyama, H. Sugiura, Y. Ando, K. Mita, M. Hamaguchi, Y. Hara, S. Kobayashi, H. Iwase, Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast*, Breast Cancer Res. Treat. 94 (2005) 11–16.

Zhang Z, Yamashita H, Toyama T, et al. Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast. Breast Cancer Res Treat 2005;94:11–6.

Zhu P, Martin E, Mengwasser J, Schlag P, Janssen KP, Go¨ttlicher M. Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis. Cancer Cell 2004;5: 455–63.